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## Sonochemical and hydrodynamic cavitation reactors for laccase/hydrogen peroxide cotton bleaching <sup>☆</sup>

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## ABSTRACT

The main goal of this work is to develop a novel and environmental-friendly technology for cotton bleaching with reduced processing costs. This work exploits a combined laccase–hydrogen peroxide process assisted by ultrasound. For this purpose, specific reactors were studied, namely ultrasonic power generator type K8 (850 kHz) and ultrasonic bath equipment Ultrasonic cleaner USC600TH (45 kHz). The optimal operating conditions for bleaching were chosen considering the highest levels of hydroxyl radical production and the lowest energy input. The capacity to produce hydroxyl radicals by hydrodynamic cavitation was also assessed in two homogenizers, EmulsiFlex<sup>®</sup>-C3 and APV-2000. Laccase nano-emulsions were produced by high pressure homogenization using BSA (bovine serum albumin) as emulsifier. The bleaching efficiency of these formulations was tested and the results showed higher whiteness values when compared to free laccase. The combination of laccase–hydrogen peroxide process with ultrasound energy produced higher whiteness levels than those obtained by conventional methods. The amount of hydrogen peroxide was reduced 50% as well as the energy consumption in terms of temperature (reduction of 40 °C) and operating time (reduction of 90 min).

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### 1. Introduction

Textile processes consume high levels of energy and water, thus innovative techniques are being developed to decrease processing time and energy consumption, while reducing the amount of chemicals involved. The use of enzymes in the Textile Industry has become an accepted strategy to overcome those issues [1]. Although the application of enzymes offers many advantages, a few drawbacks related with expensive processing costs and relatively slow reaction rates are implicated. The combination of ultrasonic energy with enzymatic treatments has become a promising approach to improve enzyme efficiency and to accelerate mass transfer during some textile processing steps, namely, desizing, scouring, bleaching, mercerizing and dyeing of textile fibres, while preserving the integrity of the fabrics [1–8]. The implementation of ultrasonic techniques to new wastewater processes for dye degradation has also been described [9].

Cavitation is the formation, growth and subsequent collapse of micro-bubbles or cavities which occur in extremely small intervals of time. It is accompanied by the release of large magnitudes of energy over a very small location producing extreme temperature and pressure gradients [10,11]. The use of ultrasound under optimum conditions can enhance diffusivity of enzymes both within and outside the vessel to influence the rates of reaction. Also ultrasound decreases transport barriers which helps to increase the enzyme:substrate ratio at target site and contribute to reduce the period of treatment by increasing enzyme availability [12–16].

Chlorine and oxygen containing oxidizing agents are used during conventional bleaching process of cellulosic fibers. When a higher whiteness is needed, it is necessary to perform multiple oxidizing treatments. Rapid bleaching with laccase–hydrogen peroxide enhances the whiteness of cotton fabrics and significantly reduces the amount of hydrogen peroxide required during subsequent chemical bleaching processes [17]. The enhanced bleaching efficiency associated with a combination of ultrasound technology, laccase and hydrogen peroxide provides or causes less fiber damage and greater uniformity of the treatment [2].

The main goal of this research is to develop a novel and environmentally beneficial technology to reduce the total processing costs of cotton bleaching consisting in the laccase bleaching of cotton assisted by acoustic cavitation (ultrasound). The specific objectives

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are increasing the laccase efficiency, decreasing the used amount of hydrogen peroxide and lowering the energy input. The dosimetric and calorimetric methods were used to characterize the different cavitation equipment used and to estimate the higher hydroxyl radical production. The effect of ultrasound on enzyme activity and stability was also assessed in this study. Additionally, laccase formulations produced by high-pressure homogenization were included in the process in order to evaluate their bleaching performance and fabric penetration level.

## 2. Materials, equipment and methods

### 2.1. Materials

Sodium phosphate dibasic, sodium phosphate monobasic, terephthalic acid, sodium hydroxide, hydrogen peroxide (50% w/v), sodium acetate trihydrate, bovine serum albumin (BSA) and 2,2-azinobis-(3-ethylbenzthiazoline)-6-sulphonate (ABTS) were purchased from Sigma–Aldrich (Spain). Acetic acid glacial was purchased from Panreac (Spain). All of these chemicals were used as supplied without any further purification. The brand of a commercial edible-grade vegetable oil used in this work was Fula Alimentar (Sovena Group, Alge's, Portugal). Laccase (EC 1.10.3.2) from ascomycete *Myceliophthora thermophila*, Novozym® 51003 (17 g protein/L, 1900 U/mL, at 50 °C), was obtained from Novozymes (Denmark). 100% of desized woven cotton fabrics and auxiliary products used on cotton bleaching experiments were supported by an industrial company.

### 2.2. Sonochemical and hydrodynamic cavitation reactors

The hydrodynamic cavitation was studied using two high-pressure homogenizers, namely EmulsiFlex®-C3 supplied from Avestin, Inc. (Canada) and APV-2000 supported by SPX (Denmark) both with maximum pressure of 2000 bar.

An ultrasonic power generator type K8, with an ultrasound frequency of 850 kHz and a maximum power output of 120 W was used for testing acoustic cavitation. This apparatus allows the selection of two action modes – pulse or continuous mode. In order to complement this equipment, an ultrasonic high-power bath Type 5/1575 it was used which was equipped with a high-performed ultrasound plan-transmitter (850 kHz), a double glass cylinder cooling system, ceramic, stainless construction and Titan-membrane. Both machines were purchased from Meinhardt Ultraschalltechnik (Germany).

An ultrasonic bath equipment Ultrasonic cleaner USC600TH (45 kHz; 120 W) was also used for cotton bleaching after pre-treatment with laccase nanoemulsions.

### 2.3. Methods

#### 2.3.1. Characterization of sonochemical and hydrodynamic cavitation reactors

**2.3.1.1. Dosimetric characterization.** The dosimetric characterization of ultrasonic equipment K8 (850 kHz, medium frequency) and sonicator bath USC600TH (45 kHz) followed the methodology referred by other authors [18–20]. The hydroxyl radicals produced by acoustic cavitation were quantified by conversion of terephthalic acid to 2-hydroxyterephthalic acid [21]. Solutions of 0.3 mM terephthalic acid (TA) were prepared in 0.1 M sodium phosphate buffer (pH 7.4) and submitted to cavitation during 30 min at 60 °C. The fluorescence of sonicated TA solutions were monitored by a Multi-mode Microplate Reader Synergy™ Mx and Gen5™ purchased from Biotek® Instruments, Inc. (USA) using a wavelength scan confirming peak emission at 425 nm from an

excitation wavelength at 315 nm. The calibration curve was plotted using standard TA solutions (0–50 mM) and 0.1 M sodium hydroxide. The hydroxyl radicals were measured at different points of the reactors considering their geometry and transducers positioning [22].

The dosimetric characterization of homogenizers followed the same methodology. The solutions of TA were submitted to cavitation under 500 bar at maximum temperatures of 35 and 55 °C for Avestin EmulsiFlex®-C3 and APV 2000, respectively.

**2.3.1.2. Calorimetric characterization.** The calorimetric characterization of all the equipment used was based on the energy measurement during time for several power inputs as it was published previously by other workers [19,23]. The measurements were performed using a Pico Technology TC-08 Analogue to Digital converter connected to a computer. The four ended k-type thermocouples (TCs) were positioned at the outer side of the ultrasonic bath and one at the center of the glass (ultrasonic power generator). For ultrasonic bath USC600TH, the TCs were placed in the same positions used to perform the calorimetric characterization (see Section 2.3.1.1). In the case of homogenizers, these measurements were made at the sample collection piece, which is not the place of higher hydroxyl radical production of the machine. However, being impossible to access the inner parts of the equipment, the amount of radicals formed were indirectly measured.

The energy profile was followed using distilled water for 30 min and the corresponding calorific power was determined following equation:

$$E = (\Delta T_{AVE} \cdot m \cdot C_p) / 1000 \quad (1)$$

where:  $E$  is the calculated energy (kJ) to raise the water temperature;  $\Delta T_{AVE}$  is equal to the difference of the final and initial temperature ( $K$ );  $m$  is the mass of the water (kg) and  $C_p$  is the heat capacity of water ( $4186 \text{ J kg}^{-1} \text{ K}^{-1}$ ); the change in temperature ( $\Delta T$ ) was calculated for each TCs positions and the final value was obtained by the mean of the five temperature sensors.

#### 2.3.2. Protein concentration and laccase activity measurements

To determine the total protein concentration of laccase solution the Bradford method using bovine serum albumin (BSA) as standard was applied [24]. The enzymatic activity of laccase was determined by oxidation of 2,2'-azinobis-(3-ethylbenzthiazoline)-6-sulphonate (ABTS) at 50 °C using acetate buffer (0.1 M) pH = 5 [25]. One unit of laccase (U) was defined as 1  $\mu\text{mol}$  of ABTS oxidized per minute. The colorimetric measurements of protein quantification and laccase activity were performed in the Helios Gamma UV-Vis spectrophotometer (Thermo Scientific, Waltham, MA, USA).

#### 2.3.3. Laccase–hydrogen peroxide bleaching of cotton fabric assisted by medium frequency ultrasound

The first step of this procedure consisted in the enzymatic pre-treatment of cotton samples with laccase assisted by medium frequency ultrasound (Ultrasonic Power Generator type K8 in the ultrasonic bath Type 5/1575 (850 kHz). For this, the cotton samples were firstly incubated with 2 U/mL of laccase from *M. thermophila* prepared in 0.1 M sodium acetate buffer (pH 5) at 50 °C for 30 min, followed by washing with distilled water at 80 °C for 10 min. In a second step, the bleaching proceeded with different hydrogen peroxide concentrations (0–8 g/L), 4 g/L NaOH and the following auxiliary products: 1 g/L anti-wrinkle, 0.5 g/L wetting agent, 1.5 g/L sequestrant, 3 g/L equalizer, 1% (o.w.f) optical brightener, assisted by a medium frequency ultrasound equipment (120 W; 850 kHz) at 55 °C for 2 h. Two different modes of action were studied: continuous mode (CM), where the ultrasound was permanently ON,

and discontinuous mode (DM), where the ultrasound was (1 break:10 pulse).

### 2.3.4. Whiteness measurement of cotton fabrics

The whiteness index ( $W^*$ ) of the treated fabrics was determined by using a reflectance measuring Datacolor apparatus at standard illuminant D65 (LAV/Spec. Excl., d/8, D65/108). For each experimental condition tested the whiteness values were measured at five different points of each sample in duplicates.

### 2.3.5. Tensile strength and elongation tests

The breaking strength and elongation tests were performed by using a tensile test machine (Hounsfield S-Series Testing; H 10 KS-UTM), according to ASTM 5035-90. The data was obtained by considering the mean of 10 replicates.

### 2.3.6. Water contact angle of bleached samples

The hydrophilicity of bleached cotton samples was observed by measuring the water contact angle using a Dataphysics instrument (Filderstadt, Germany) and the OCA20 software (Germany). The data obtained was the result of the average of 10 different points of each sample. The water droplet volume was set as 5  $\mu\text{L}$  using a Hamilton 500  $\mu\text{L}$  syringe. Conditions for measurement were selected as ellipse fitting. The sample images were captured by a SCA-20 live camera at the first second after the water dropped on samples.

### 2.3.7. Laccase nanoemulsions prepared by homogenization

The laccase nanoemulsions, containing 99.5%  $\text{H}_2\text{O}$ /0.5% oil (vegetable oil), were prepared using 10 g/L of laccase, 5 g/L BSA in acetate buffer pH 5 to form an oil-in-water (O/W) emulsion. The two phases once mixed were homogenized (39 cycles – 13 min) using the homogenizer EmulsiFlex-C3 under high pressure (500 bar).

### 2.3.8. Laccase formulations–hydrogen peroxide bleaching of cotton fabric assisted by low frequency ultrasound

Samples of cotton were previously pretreated using laccase nanoemulsions following the experimental conditions referred at Section 2.3.3. The cotton fabrics were bleached afterwards using 8 g/L  $\text{H}_2\text{O}_2$  and specific auxiliary products. The bleaching was carried out at 80  $^\circ\text{C}$  for 1 h, in an ultrasound bath sonicator (VWR Ultrasonic cleaner) with a fixed frequency 45 kHz and power intensity at 120 W.

## 3. Results

### 3.1. Dosimetric and calorimetric characterization of cavitation equipment

The reactors were characterized via dosimetric and calorimetric procedures as described in Section 2.3.1 in order to evaluate which equipment was able to produce the highest level of hydroxyl radicals, an essential condition to speed up the bleaching reactions and to improve the protein formulations production [2,26].

In the reactor used for bleaching (ultrasonic power generator K8), these two procedures were performed using different power intensities (30, 60, 90 and 120 W) at 850 kHz. The results are presented in Fig. 1 which shows an increase of the energy deposition with the power input. As hydroxyl radical indicator, the TA conversion to HTA was measured and the obtained yield of conversion demonstrated an almost linear production of radicals with increasing power intensity. Comparing both modes of ultrasound action, continuous and discontinuous, it was possible to verify that when the sonication was performed continuously, the level of energy involved on the aqueous system was considerably higher.

The performance of the four cavitation equipments used in this work were compared in terms of hydroxyl radical production versus energy input. From all, the highest hydroxyl radical production was verified for sonicator type K8 (850 kHz) followed by ultrasonic bath 45 kHz (Fig. 2).

To our knowledge this was the first characterization of hydroxyl production by the homogenization method. The two homogenizers tested demonstrated the lowest hydroxyl yield and the highest energy levels when compared with acoustic reactors. In the medium frequency equipment, the irradiation of water with ultrasound leads to the breakdown, or sonolysis, of the liquid resulting in the formation of hydroxyl and hydrogen radicals while in homogenizers the phenomenon involves large pressure losses at the gap inlet and outlet, as well as at the gap itself, causing the hydrodynamic cavitation in the homogenization valve [12,14,27]. While acoustic cavitation is the result of the ultrasound passage through the medium, hydrodynamic cavitation results from the velocity variation in the flue due to the changing geometry of the fluid flow path. The hydrodynamic cavitation phenomenon involved in high-pressure homogenizers is based on the pumping of the liquid at very high pressure through a constriction which converts its pressure energy into kinetic energy. At extremely high kinetic energy levels, the pressure reduces below the liquid vapor

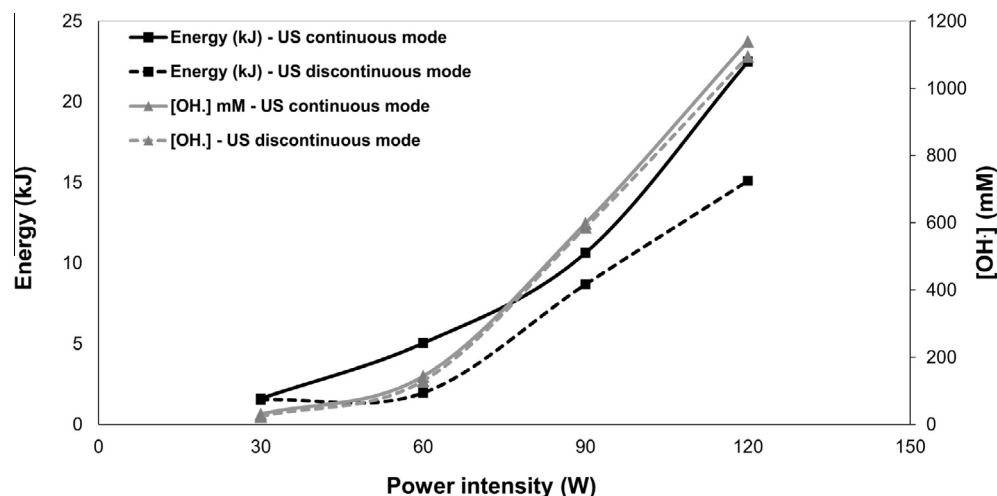


Fig. 1. Variation of  $\cdot\text{OH}$  radicals rate (mM) and input energy (kJ) with ultrasound power intensity (W), for medium frequency equipment using continuous (—■) and discontinuous (---■) mode.

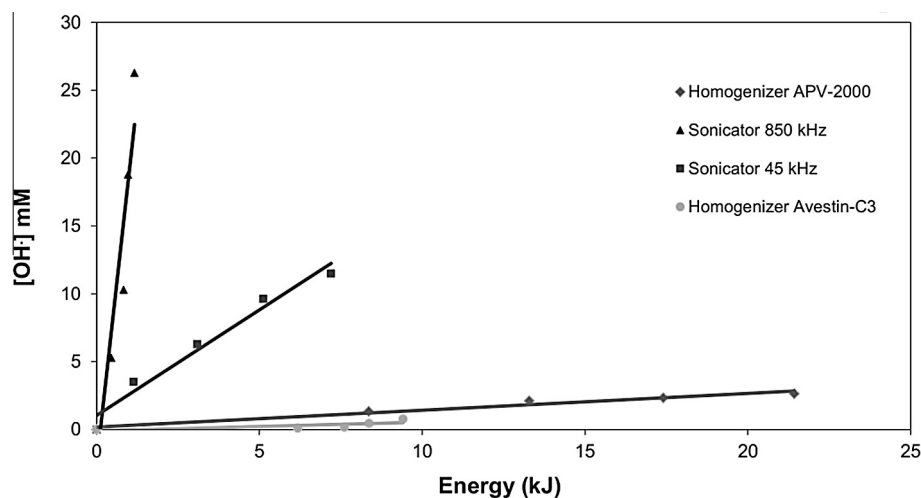


Fig. 2. Formation of hydroxyl radicals (mM) in function of energy induced (kJ) on the 4 systems used (homogenizer APV-2000, homogenizer Avestin-C3, sonicator type K8 850 kHz and bath sonicator 45 kHz).

pressure generating cavitation [28,29]. In spite of the difference between the two mechanisms of cavitation, the bubble behavior show similar trends with the variation of experimental parameters. Several reports appear in literature confirming that hydrodynamic cavitation is more energy efficient than acoustic cavitation [12]. Low energy effectiveness of acoustic cavitation can be mainly attributed to the fact that most of the energy delivered by the transducer is wasted and moreover the active cavitation zone is concentrated very near to the transducer surface, which can be a limitation for the scale up of acoustic transducers. On the other hand, hydrodynamic cavitation reactors offer much larger activate cavitating zones, which can also be adjusted based on the geometry modification of the cavitation chamber [30]. Results from Fig. 2 corroborate these assumptions. High frequency reactors produced high levels of radicals corresponding to low energy input. In the case of hydrodynamic cavitation (homogenizers), some of the energy produced is dissipated and the radicals production is not concentrated in specific points of the reactor, thus the hydroxyl radicals level attained is lower when compared with acoustic cavitation [28]. The characterization of both homogenizers was performed at 500 bar which could impair the collapse pressure due to single cavity. However, further equivalent intensities can be suitable achieved for the desired transformation, by increasing the pressure of the reactors. The different hydroxyl radical levels attained for both homogenizers can be explained by their different

geometry. Due to the horizontal flow inside the Avestin, there is a flotation of the liquid and thus a blocking of the gap can occur frequently. This is avoided working with the APV equipment because the preparation passes the homogenizer in a vertical way. The different construction of the homogenizers leads to a different homogenization performance. The results obtained confirmed previous reports by others where the greatest sonochemical efficiency in producing hydroxyl radicals was attained for the highest applied frequency [18].

### 3.2. Influence of ultrasound on laccase activity

The enhancement of enzymatic textile processes accomplished by the incorporation of ultrasound technology has been widely reported, however the effect of ultrasound on enzymes is not well known and many contradictory results have been observed when enzymes are treated with high-intensity ultrasound.

Enzymes are easily denaturated by changes on the environment conditions such as temperature, pressure, shear stress, pH and ionic strength. As mentioned in literature, the radicals produced by cavitation phenomenon are highly reactive and can promote several physical and chemical reactions. These reactions, together with the heat generated, can interfere with enzyme activity and stability. To evaluate this, the laccase activity was measured at different times of cotton incubation in presence of medium frequency ultrasound

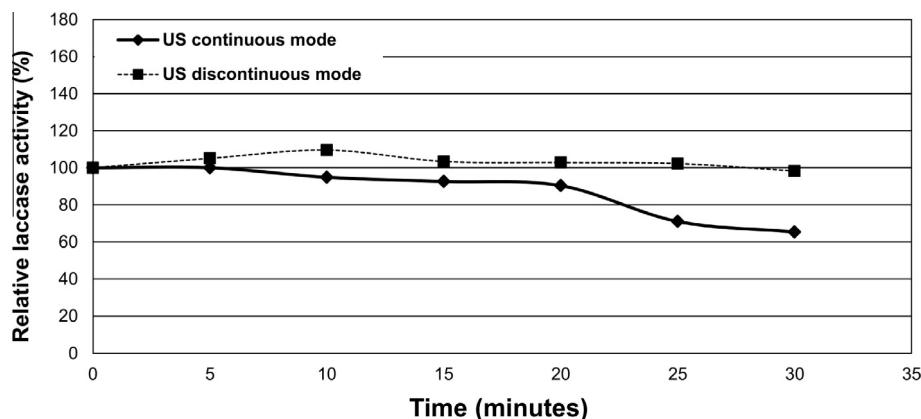


Fig. 3. Laccase activity during cotton pre-treatment in the presence of ultrasounds (850 kHz; 120 W) using 0.1 M sodium acetate buffer (pH 5), 50 °C for 30 min. 100% of enzyme activity corresponds to 1900 U/mL (1 U is defined as 1  $\mu$ mol of ABTS oxidized per minute).

and the results are presented in Fig. 3. It can be drawn that laccase activity decreases 40% after 30 min of continuous cavitation. The vibrational motions induced by ultrasound likely enhance enzyme unfolding causing denaturation and activity loss. Moreover, the increase in temperature and the chemical modifications arising from the water sonolysis and radical formation are also important factors that may affect enzyme activity and stability. It is our assumption that loss of laccase activity under ultrasonication can be due to the reaction of the protein with the radicals produced during cavitation (hydroxyl radicals, superoxide and peroxide) that may act as potential cross-linking agents promoting protein aggregation. This theory was already confirmed by several authors that observed the formation of macromolecular aggregates of laccase after ultrasound treatment [18]. According to previous studies these aggregates could be produced by the exposure of the cysteine residues from proteins to the high reactive generated superoxide radicals formed in the sonication of water under air, thus leading to the formation of disulphide bonds between proteins [31]. Differently, discontinuous cavitation influenced positively this property. In this case, the results show a slight increase of activity in the first 10 min of cavitation, probably due to the effects on the formation and dissociation of enzyme-substrate complex.

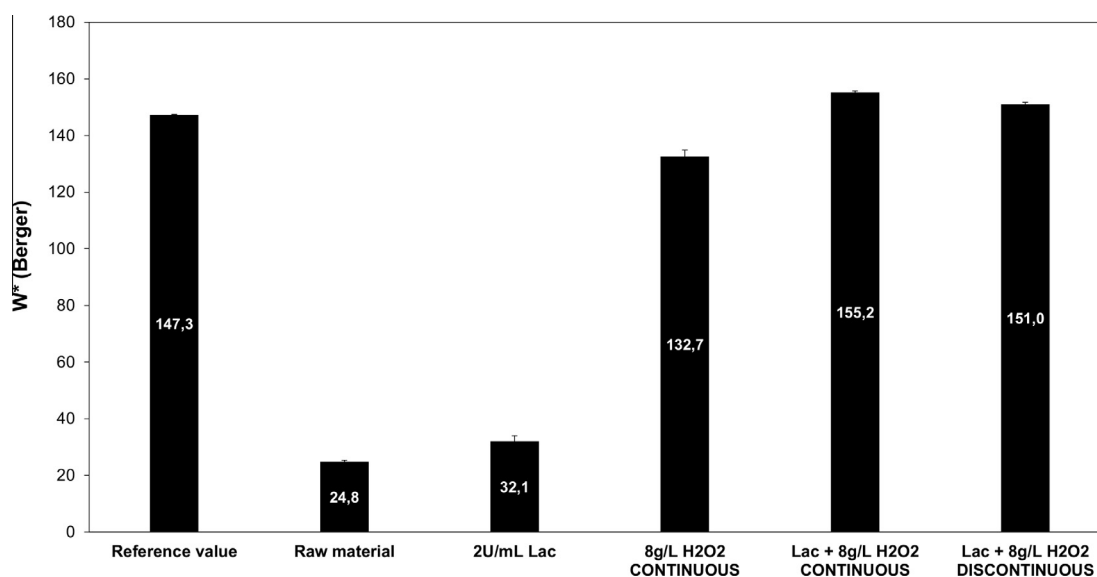
Though the laccase activity decreased along the process of enzymatic bleaching assisted by ultrasound, high levels of activity were still maintained most of the processing time without disturbing the final whiteness levels.

### 3.3. Laccase–hydrogen peroxide bleaching of cotton fabric assisted by medium frequency ultrasound

The optimized conditions of the combined laccase–hydrogen peroxide bleaching process (Section 2.3.3.) were tested now on cotton fabric samples aiming to improve the overall bleaching efficiency by means of ultrasonic energy. The effect of ultrasound was tested at different power levels (30–120 W) with medium ultrasonic frequency (850 kHz) and the presented results were obtained using the higher power input (120 W). Fig. 4 shows the effectiveness of ultrasound on the enhancement of the combined laccase–hydrogen peroxide bleaching process which may be explained by the cavitation and heating effects.

The acoustic cavitation occurring near the solid surface will generate micro jets which will enhance the mass transfer resulting in an increased diffusion of solute through the pores of the fabric. In the case of sonication, localized temperature raise and swelling effects may also improve the diffusion. In this heterogeneous solid/liquid system, the collapse of the cavitation bubbles results in significant structural and mechanical effects. Cavitation (growth and explosive collapse of microscopic bubbles) can generate “hot spots” i.e. localized high temperature and shock waves producing high pressure capable of breaking chemical bonds [32]. Collapse near the surface produces an asymmetrical inrush of the fluid to fill the void forming a liquid jet targeted at the surface. These jets activate the solid substrate and increase the mass transfer to the surface by the disruption of the interfacial boundary layers as well as dislodging the reaction products exposing new sites for oxidation. At the same time and due to the high temperature and pressure inside the bubbles in the strong collapse, water vapor is dissociated and chemical products such as OH<sup>•</sup>, O<sup>•</sup> and H<sup>•</sup>, as well as H<sub>2</sub>O<sub>2</sub> are created and are responsible for the bleaching improvements. At this point, laccase oxidizes the phenolic compounds which are more easily susceptible to oxidation with peroxide and thus more easily degraded and removed from cotton surface [17]. Laccase pre-treatment was essential to reach whiteness values higher than reference. This was confirmed by bleaching cotton fabrics without any pretreatment using 8 g/L hydrogen peroxide. The action of oxidant agent, even assisted by ultrasound, was not enough to impart the required whiteness. Moreover, ultrasound provides the additional effect of re-aggregation of enzyme molecules increasing enzyme activity and leading to a faster motion and stirring effects in the combined laccase–hydrogen/ultrasound assisted bleaching. Thus, ultrasound cavitation increased the surface area accessible for reaction through efficient mixing and enhanced transport and provided additional laccase activation [12].

Our findings suggest that the whiteness levels obtained by applying continuous and discontinuous ultrasound are different due to the different reached temperatures in each mode (Fig. 1). The temperature on the reactor is also an important factor to be taken into account for bleaching efficiency. The continuous mode afforded higher levels of energy input at 60 °C than the discontinuous mode. This corresponded to a more efficient bleaching imparted by the



**Fig. 4.** Whiteness of cotton samples bleached by combined laccase–hydrogen peroxide/ultrasound process (reference value: sample treated with the conventional conditions – 8 g/L H<sub>2</sub>O<sub>2</sub>, 95 °C for 270 min; raw material: sample without any treatment; 2 U/mL Lac: pre-treatment using 2 U/mL of laccase in acetate buffer 0.1 M, pH = 5 at 50 °C for 30 min assisted by ultrasound (850 kHz; 120 W), washing with distilled water at 80 °C for 10 min; 8 g/L H<sub>2</sub>O<sub>2</sub> CONTINUOUS: non pre-treated sample bleached with 8 g/L H<sub>2</sub>O<sub>2</sub> assisted by ultrasound (850 kHz; 120 W) at 55 °C for 2 h; Lac + 8 g/L H<sub>2</sub>O<sub>2</sub>: bleached sampled using combined laccase/hydrogen peroxide system); all the experiments were carried out using ultrasounds at 850 kHz with 120 W, continuous and discontinuously.

swelling effects and the oxidant agent diffusion into the fiber. From Fig. 4 it is also possible to conclude that using the same oxidant agent concentration, samples bleached using the combined system presented higher whiteness than reference samples. The tested system allowed us to conclude that it is possible to decrease the chemical consumption decreasing therefore the final process costs.

Different oxidant agent concentrations were tested for the combined bleaching system and the results are presented in Fig. 5. As expected, the results show a proportional increase of the whiteness values with the increase of oxidant concentration. Moreover, it can be depicted that a low concentration of hydrogen peroxide can be applied (4 g/L) to obtain whiteness levels similar to reference sample. Thus, due to the several benefits already discussed above, ultrasound contributed to the reduction of the amount of hydrogen peroxide required for bleaching purposes attaining an efficient and profitable bleaching process.

### 3.3.1. Tensile strength and elongation evaluation

One aspect to consider when chemical and mechanical treatments are considered is the fabric's tensile strength loss. This is a parameter that would restrict the subsequent textile processes as well as the final garment performance. In order to evaluate if the bleaching process could affect the tensile strength of textile materials, we measured the breaking strength (Fig. 6A) and the elongation (Fig. 6B) of the bleached samples. We can conclude that the breaking strength of sonicated samples is higher than reference samples, indicating that enzymatic pre-treatment and sonication did not impair the mechanical behavior of fabric samples. Moreover, the quality and integrity of fabrics was maintained as it can be also confirmed by the elongation results (Fig. 6B).

### 3.3.2. Hydrophilicity of bleached fabric sample

After bleaching it is pertinent that fabrics become more hydrophilic and able to be transformed in the subsequent phases of textile processing like dyeing or printing. Hence, we measured the water contact angle of samples after laccase pre-treatment and after combined bleaching. The results from Table 1 show that after bleaching the samples became extremely hydrophilic, as expected (water contact angle  $\approx 0$ ). The hydrophobic character conferred by the phenolics is eliminated as these compounds are oxidized by laccase and removed from fabrics surface during the combined bleach process assisted by ultrasound.

### 3.4. Laccase/BSA formulations production assisted by hydrodynamic cavitation for bleaching purposes

The production of protein nanoemulsions was performed in order to increase laccase efficiency mainly due to increasing stability during the sonication period. Different methodologies are used for nanoparticles production. Since the use of ultrasound increases the risk of samples contamination with metals from the probe and of protein denaturation, the high pressure homogenization was assessed in this work as an alternative for nanoemulsions production. Taking advantage of BSA emulsifying properties, nanoemulsions made of laccase and BSA were synthesized by high pressure homogenization, using different concentrations of both proteins, forming O/W (0.5/99.5) emulsions. Fig. 7a) represents the physical appearance of native laccase and its nanoemulsion where the proteins, BSA and laccase, are highly dispersed in the oil as nano-order dispersions. The formation and characterization of proteinaceous nanoemulsions using high pressure homogenization has been extensively studied [33–37]. In this process, several factors have been taken into account, namely pressure, temperature, number of passes and flow rate. In a biphasic system, emulsification must occur during the microscopic dispersion of the nonaqueous phase into the aqueous protein solution [38]. However, the mechanism of nanoemulsions formation is not completely explained by the homogenization process if self, instead other aspects like the nature of protein and hydroxyl radical production during homogenization process might be considered. The mechanism of nanoemulsions production considered the formation of  $\cdot\text{OH}$  and  $\cdot\text{H}$  radicals, that formed  $\text{H}_2$  and  $\text{H}_2\text{O}_2$ , and, in the presence of  $\text{O}_2$  formed  $\cdot\text{HO}_2$  Hydroxyl, superoxide and peroxide radicals are all potential protein cross-linking agents. The cysteine residues, a key requirement for stable nanoemulsions formation, are present in BSA in a much higher extent (aa 35) than in laccase (aa 8), being oxidized by the superoxide radical. They are held together by protein cross-linking through disulfide linkages from cysteine oxidation [38,39]. Together with the cysteine residues of the proteins, the amount of hydrophobic amino acids plays also a significant role on the nanoemulsion production [40]. The morphology of the produced nanoemulsions, determined by transmission microscopy (STEM) is presented in Fig. 7b). According to this figure, the proteinaceous nanoemulsions were spherical with a regular surface. This morphology allows a higher protein stabil-

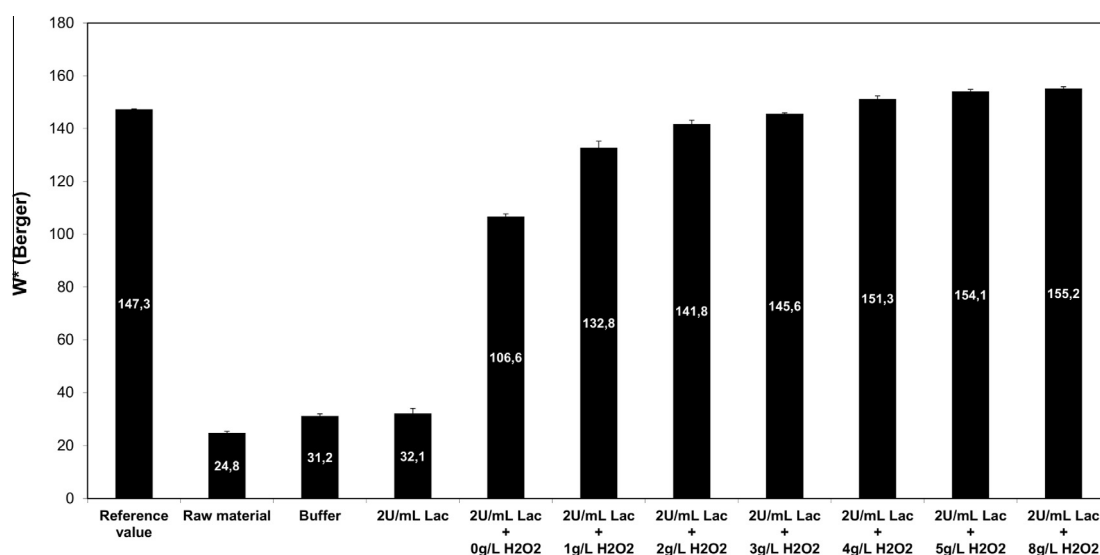
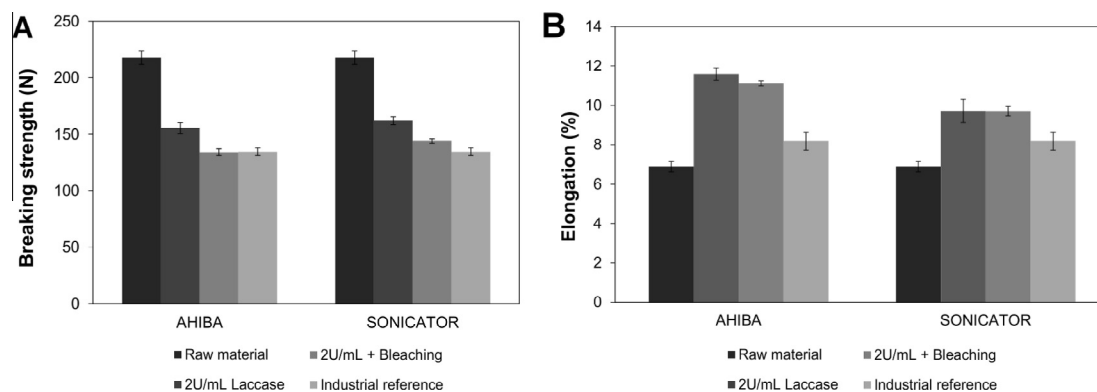


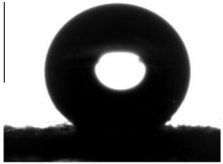

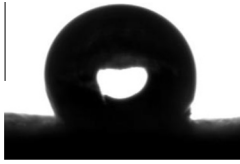

Fig. 5. Whiteness of cotton samples using different hydrogen peroxide concentrations (reference value: sample treated with the conventional conditions – 8 g/L  $\text{H}_2\text{O}_2$ ; 95 °C for 270 min; raw material: sample without any treatment; US laccase pre-treatment: 2 U/mL of enzyme at 50 °C for 30 min assisted by ultrasound (850 kHz; 120 W), washing with distilled water at 80 °C for 10 min; US bleaching: 0–8 g/L  $\text{H}_2\text{O}_2$  at 55 °C for 2 h (850 kHz; 120 W), washing with distilled water.

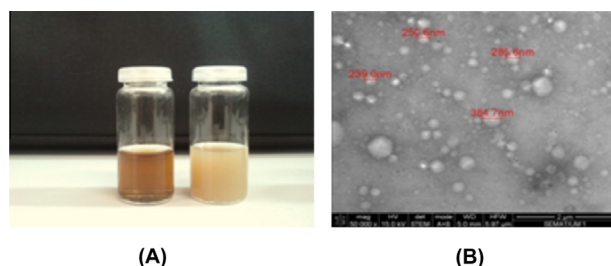


**Fig. 6.** (A) Breaking strength (N) and (B) elongation (%) values of cotton fabrics treated using combined laccase/ultrasound process.

**Table 1**

Water contact angle of bleached fabric samples; raw fabric: sample without any treatment; reference: sample bleached with a conventional method using 8 g/L H<sub>2</sub>O<sub>2</sub>, 95 °C for 270 min; laccase pre-treatment: 2 U/mL of laccase at 50 °C for 30 min; US bleaching: 4 g/L H<sub>2</sub>O<sub>2</sub>, 55 °C for 2 h; both laccase pre-treatment and bleaching were assisted by ultrasound (K8 850 kHz; 120 W).

	Raw fabric	Reference	Laccase pre-treatment	US bleaching
Water contact angle (°)	 153.62 ± 4.46	 ≈0	 139.27 ± 7.56	 ≈0



**Fig. 7.** (A) Laccase nanoemulsions aspect after homogenization, Left: Free laccase, Right: Laccase formulation: 5 g/L BSA + 10 g/L laccase + 0.5% oil; (B) transmission electron microscopy (STEM) of laccase/BSA formulation [43].

ization, since a minimum contact with water is provided, requiring at the same time a small amount of surface-active agent for stabilization [41]. STEM technique showed an average particle size around 250 nm, corresponding to the values obtained by dynamic light scattering (DLS) measurements (data not shown).

### 3.5. Laccase/BSA-hydrogen peroxide bleaching of cotton fabric assisted by low frequency ultrasound

In order to reduce the overall costs of the process, the bleaching of cotton fabrics was also tested using laccase/BSA nanoemulsions and assisted by low frequency ultrasound. From Table 1, two important

conclusions have to be considered. Firstly, both free and formulated laccase pre-treatment increased the whiteness values. Secondly, the application of formulated laccase in a pretreatment stage of the process is determinant to increase the whiteness levels. It is interesting to notice that both free and laccase formulations were able to produce similar effect on cotton fabrics, however after bleaching, the whiteness values were higher when formulated laccase was used. The ability of laccase to oxidize phenolic compounds naturally existing at cotton surface was effectively enhanced by using the enzyme formulation. Smaller and stable emulsions were able to diffuse easily on cotton, oxidizing a higher extent of phenolics that were further removed by a bleaching process. Additionally, some of the laccase own natural color which is adsorbed to cotton surface and many times inefficiently removed would also impair an efficient bleaching. The better performance of the nanoemulsions on cotton bleaching can also be attributed to the increase of laccase operating stability in the formulation when compared to the free enzyme [42].

Apart of operating stability, the storage stability was also of prime importance. We have tested if the formulation was able to efficiently oxidize phenolics on cotton after 4 weeks of storage (Table 2). The whiteness of cotton fabrics bleached after pre-treatment with this formulation was slightly higher when compared with the time zero formulation. From this, it can be stated that laccase is efficiently stabilized by BSA/laccase nanoemulsions.

By applying low frequency ultrasound it was possible to improve the bleaching efficiency when laccase nanoemulsions were

**Table 2**

Whiteness Berger (W\*) level for optical bleaching of samples pre-treated with laccase formulation.

Samples	Whiteness (W*) after pre-treatment	Whiteness (W*) after combined bleaching (time zero formulation)	Whiteness (W*) after combined bleaching (4 weeks of storing)
Raw cotton fabric	27.2	160.8	–
Control (acetate buffer)	30.9	163.0	–
Free laccase	35.1	151.3	–
Laccase formulation	35.2	170.1	172.7

used on fabrics pre-treatment. When the pre-treatment was performed with free laccase, the medium frequency equipment is still required to attain higher bleaching effects.

#### 4. Conclusions

The use of medium frequency ultrasound (850 kHz, 120 W) improved the cotton bleaching using a combined laccase–hydrogen process. Compared with conventional methods, this approach allowed to increase whiteness levels and to reduce the amount of hydrogen peroxide in 50%. The energy consumption in terms of temperature (reduction of 40 °C) and operating time (reduction of 90 min) was also reduced.

The hydroxyl radical production by high pressure homogenization allowed producing more stable laccase nanoemulsions for bleaching improvement. In this case, the mass transfer from the bulk solution to the fiber was not only enhanced by ultrasonic cavitation but also by laccase emulsions shape, facilitating fiber diffusion.

This investigation has confirmed that ultrasound power is beneficial to the cotton bleaching by the combined laccase/hydrogen peroxide process. This tandem technology, in addition to its economic advantage of saving energy by lowering hydrogen peroxide, temperature and incubation time, has a much lower environmental impact compared with the conventional process.

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#### References

- [1] C. Karaboga, A.E. Korlu, K. Duran, M.I. Bahtiyari, Use of ultrasonic technology in enzymatic pretreatment processes of cotton fabrics, *Fibers and Textiles in Eastern Europe* 15 (2007) 97–100.
- [2] C. Basto, T. Tzanov, A. Cavaco-Paulo, Combined ultrasound-laccase assisted bleaching of cotton, *Ultrasonics Sonochemistry* 14 (2007) 350–354.
- [3] A. Abou-Okeil, A. El-Shafie, M.M. El Zawahry, Ecofriendly laccase–hydrogen peroxide/ultrasound-assisted bleaching of linen fabrics and its influence on dyeing efficiency, *Ultrasonics Sonochemistry* 17 (2010) 383–390.
- [4] M. Parvinzadeh, N. Memari, M. Shaver, B. Katozian, S. Ahmadi, I. Ziadi, Influence of ultrasonic waves on the processing of cotton with cationic softener, *Journal of Surfactants and Detergents* 13 (2010) 135–141.
- [5] V.S. Moholkar, M.M.C.G. Warmoeskerken, Investigations in mass transfer enhancement in textiles with ultrasound, *Chemical Engineering Science* 59 (2004) 299–311.
- [6] A. Hebeish, S. Sharaf, M.M.A. El-Hady, Ultrasound aided KMnO<sub>4</sub>-acid systems for bleaching linen fabric, *Carbohydrate Polymers* 83 (2011) 1370–1376.
- [7] S.J. McNeil, R.A. McCall, Ultrasound for wool dyeing and finishing, *Ultrasonics Sonochemistry* 18 (2011) 401–406.
- [8] M. Vouters, P. Rumeau, P. Tierce, S. Costes, Ultrasounds: an industrial solution to optimise costs, environmental requests and quality for textile finishing, *Ultrasonics Sonochemistry* 11 (2004) 33–38.
- [9] S. Vajnhandl, A. Majcen Le Marechal, Ultrasound in textile dyeing and the decolouration/mineralization of textile dyes, *Dyes and Pigments* 65 (2005) 89–101.
- [10] P.R. Gogate, A.B. Pandit, Engineering design method for cavitation reactors: I. Sonochemical reactors, *AIChE Journal* 46 (2000) 372–379.
- [11] P.R. Gogate, A.B. Pandit, Sonochemical reactors: scale up aspects, *Ultrasonics Sonochemistry* 11 (2004) 105–117.
- [12] P.R. Gogate, A.M. Kabadi, A review of applications of cavitation in biochemical engineering/biotechnology, *Biochemical Engineering Journal* 44 (2009) 60–72.
- [13] P.R. Gogate, V.S. Sutkar, A.B. Pandit, Sonochemical reactors: important design and scale up considerations with a special emphasis on heterogeneous systems, *Chemical Engineering Journal* 166 (2011) 1066–1082.
- [14] L. Csoka, S.N. Katekhaye, P.R. Gogate, Comparison of cavitation activity in different configurations of sonochemical reactors using model reaction supported with theoretical simulations, *Chemical Engineering Journal* 178 (2011) 384–390.
- [15] P.R. Gogate, Cavitation reactors for process intensification of chemical processing applications: A critical review, *Chemical Engineering and Processing: Process Intensification*, 47 (2008) 515–527.
- [16] B. Kwiatkowska, J. Bennett, J. Akunna, G.M. Walker, D.H. Bremner, Stimulation of bioprocesses by ultrasound, *Biotechnology Advances* 29 (2011) 768–780.
- [17] T. Tzanov, C. Basto, G.M. Gübitz, A.C. Paulo, Laccases to improve the whiteness in a conventional bleaching of cotton, *Macromolecular materials and engineering* 288 (2003) 807–810.
- [18] C. Basto, C.J. Silva, G. Gübitz, A. Cavaco-Paulo, Stability and decolorization ability of *trametes villosa* laccase in liquid ultrasonic fields, *Ultrasonics Sonochemistry* 14 (2007) 355–362.
- [19] C. Little, M. El-Sharif, M.J. Hefher, The effect of solution level on calorific and dosimetric results in a 70 kHz tower type sonochemical reactor, *Ultrasonics Sonochemistry* 14 (2007) 375–379.
- [20] G.J. Price, F.A. Duck, M. Digby, W. Holland, T. Berryman, Measurement of radical production as a result of cavitation in medical ultrasound fields, *Ultrasonics Sonochemistry* 4 (1997) 165–171.
- [21] C. Little, M. El-Sharif, M.J. Hefher, The effect of solution level on calorific and dosimetric results in a 70 kHz tower type sonochemical reactor, *Ultrasonics Sonochemistry* 14 (2007) 375–379.
- [22] S. Perincek, A.E. Uzgur, K. Duran, A. Dogan, A.E. Korlu, İ.M. Bahtiyari, Design parameter investigation of industrial size ultrasound textile treatment bath, *Ultrasonics Sonochemistry* 16 (2009) 184–189.
- [23] R. Silva, H. Ferreira, C. Little, A. Cavaco-Paulo, Effect of ultrasound parameters for unilamellar liposome preparation, *Ultrasonics Sonochemistry* 17 (2010) 628–632.
- [24] M.M. Bradford, A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding, *Analytical Biochemistry* 72 (1976) 248–254.
- [25] R.E. Childs, W.G. Bardsley, The steady-state kinetics of peroxidase with 2,2'-azino-di-(3-ethyl-benzthiazoline-6-sulphonic acid) as chromogen, *The Biochemical Journal* 145 (1975) 93–103.
- [26] R. Silva, H. Ferreira, N.G. Azoia, U. Shimanovich, G. Freddi, A. Gedanken, A.C. Paulo, Insights on the mechanism of formation of protein microspheres in a biphasic system, (2012).
- [27] A. Håkansson, L. Fuchs, F. Innings, J. Revstedt, B. Bergenstahl, C. Trägårdh, Visual observations and acoustic measurements of cavitation in an experimental model of a high-pressure homogenizer, *Journal of Food Engineering* 100 (2010) 504–513.
- [28] P.R. Gogate, A.B. Pandit, A review and assessment of hydrodynamic cavitation as a technology for the future, *Ultrasonics Sonochemistry* 12 (2005) 21–27.
- [29] S.I. Nikitenko, C. Le Naour, P. Moisy, Comparative study of sonochemical reactors with different geometry using thermal and chemical probes, *Ultrasonics Sonochemistry* 14 (2007) 330–336.
- [30] P.R. Gogate, Cavitation: an auxiliary technique in wastewater treatment schemes, *Advances in Environmental Research* 6 (2002) 335–358.
- [31] S. Avivi, A. Gedanken, S–S bonds are not required for the sonochemical formation of proteinaceous microspheres: The case of streptavidin, *Biochemical Journal* 366 (2002) 705–707.
- [32] K.S. Suslik, F.J.-J. Toublan, S.A. Boppart, D.L. Marks, Surface modified protein microparticles, in, 2007.
- [33] A. Patidar, D.S. Thakur, P. Kumar, J. Verma, A review on novel lipid based nanocarriers, *International Journal of Pharmacy and Pharmaceutical Sciences* 2 (2010) 30–35.
- [34] S. Liedtke, S. Wissing, R.H. Müller, K. Mäder, Influence of high pressure homogenisation equipment on nanodispersions characteristics, *International Journal of Pharmaceutics* 196 (2000) 183–185.
- [35] R.H. Müller, K. Mäder, S. Gohla, Solid lipid nanoparticles (SLN) for controlled drug delivery – a review of the state of the art, *European Journal of Pharmaceutics and Biopharmaceutics* 50 (2000) 161–177.
- [36] J. Rao, D.J. McClements, Formation of flavor oil microemulsions, nanoemulsions and emulsions: influence of composition and preparation method, *Journal of Agriculture and Food Chemistry* 59 (2011) 5026–5035.
- [37] A. Patidar, D.S. Thakur, P. Kumar, J. Verma, A review on novel lipid based nanocarriers, *International Journal of Pharmacy and Pharmaceutical Sciences* 2 (2010) 30–35.
- [38] A. Gedanken, Preparation and properties of proteinaceous microspheres made sonochemically, *Chemistry – A European Journal* 14 (2008) 3840–3853.
- [39] S. Avivi, A. Gedanken, The preparation of avidin microspheres using the sonochemical method and the interaction of the microspheres with biotin, *Ultrasonics Sonochemistry* 12 (2005) 405–409.
- [40] K.P. Bidyut, S.P. Moulik, Uses and applications of microemulsions, *Current Science* 80 (2001) 990–1001.
- [41] E. Dickinson, Interfacial interactions and the stability of oil-in water emulsions, *Pure and Applied Chemistry* 64 (1992) 1721–1724.
- [42] J. Rangsanarid, K. Fukada, Factors affecting the stability of O/W emulsion in BSA solution: stabilization by electrically neutral protein at high ionic strength, *Journal of Colloid and Interface Science* 316 (2007) 779–786.
- [43] M. Martins, C. Silva, A. Cavaco-Paulo, Interfacial laccase stabilization for cotton bleaching, *Adhesion Aspects in Modern Enzymology: Special Issue of the Journal of Adhesion Science and Technology* accepted (2012).